# Efficiency in Peptide Coupling: 1-Hydroxy-7-azabenzotriazole *vs* 3,4-Dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine†

Louis A. Carpino* and Ayman El-Faham‡

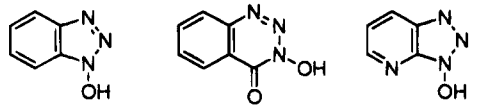
*Department of Chemistry, Box 34510, University of Massachusetts, Amherst, Massachusetts 01003-4510*

Fernando Albericio§

*Millipore Corporation, 75A Wiggins Avenue, Bedford, Massachusetts 01730*

*Received January 3, 1995*

1-Hydroxybenzotriazole (HOBt, **1**), either as an additive in combination with a carbodiimide or built into a variety of stand-alone reagents (BOP, HBTU, etc.), has become widely adopted for peptide coupling to avoid loss of chirality and side product formation since König and Geiger first announced its use in 1970[1]. In the König and Geiger study, over 30 other *N*-hydroxy compounds were described, and only one, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (HODhbt, **2**), proved to be generally superior to HOBt.



**1** **2** **3**

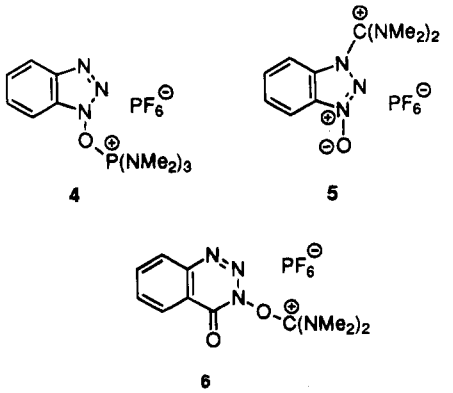
Unfortunately, as pointed out by König and Geiger and subsequently confirmed by others,[2] the use of HODhbt is circumscribed due to inherent side reactions, which have limited its widespread adoption. On the other hand, protected amino acid esters derived from HODhbt, if prepared in a manner which avoids the presence of contaminants,[3] can be advantageous where their increased reactivity relative to their OBt analogs is desired.

**Table 1. Effect of Solvent and Additive on the Preservation of Configuration during Formation of Z-Phg-Pro-$NH_2$ via Carbodiimide Coupling[a,b]**

| coupling reagent | solvent | yield, % | DL, % |
|---|---|---|---|
| EDC/HOAt (DCC)[c] | DMF | 81.4 (83.0) | 7.0 (3.3) |
| EDC/HOAt (DCC)[c] | TFE/TCM | 50.1 (71.3) | <0.1 (0.7) |
| EDC/HODhbt (DCC)[c] | DMF | 80.4 (81.3) | 24.6 (16.7) |
| EDC/HODhbt (DCC)[c] | TFE/TCM | 51.2 (71.5) | 1.2 (1.4) |
| EDC/HOBt (DCC)[c] | DMF | 81.9 (89.2) | 10.1 (9.3) |
| EDC/HOBt (DCC)[c] | TFE/TCM | 48.0 (72.1) | <0.1 (0.9) |

[a] Coupling reactions were carried out as described in footnotes *a*–*d*, Table 2. [b] Test peptide Z-Phg-Pro-$NH_2$ has been described elsewhere (Wenschuh, H.; Beyermann, M.; Haber, H.; Seydel, J. K.; Krause, E.; Bienert, M.; Carpino, L. A.; El-Faham, A.; Albericio, F. *J. Org. Chem.* **1995**, *60*, 405). [c] Figures in parentheses are those for runs in which DCC was used in place of EDC. For all such runs with DCC, 1 equiv of TMP was added. When EDC·HCl was used, 1 equiv of TMP was also added. In this case, the results were similar to those observed for EDC itself.

Phosphonium[4] and ammonium (guanidinium)[5] coupling reagents **4** and **5**, respectively, derived from HOBt are becoming more and more popular, and recently, the analogous reagent HDTU, **6**, derived from HODhbt was described by Knorr and co-workers,[6] who stated that, while less loss of chirality accompanies its use, "it is



**4** **5**

**6**

recommended only in critical cases because of the danger of side reactions." [6] More recently, Sakakibara and co-

†Abbreviations not defined in the text: BOP = (benzotriazolyloxy)-tris(dimethylamino)phosphonium hexafluorophosphate; DCC = dicyclohexylcarbodiimide; DIEA = diisopropylethylamine; DMF = dimethylformamide; EDC = 1-ethyl-3-(3′-(dimethylamino)propyl)carbodiimide; HAPyU = *O*-(7-azabenzotriazol-1-yl)-1,1:3,3-bis(tetramethylene)uronium hexafluorophosphate; HATU = *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU = *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HDTU = *O*-(3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl)-1,1:3,3-tetramethyluronium hexafluorophosphate; Phg = α-phenylglycine; TMP = collidine = 2,4,6-trimethylpyridine; Z = benzyloxycarbonyl.

‡On leave from the Department of Chemistry, Faculty of Science, Alexandria University, Alexandria, Egypt.

§Current address: Department of Organic Chemistry, University of Barcelona, E-08028 Barcelona, Spain.

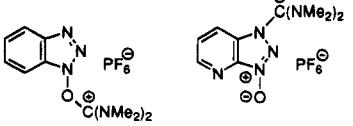
(1) (a) König, W.; Geiger, R. *Ber. Dtsch. Chem. Ges.* **1970**, *103*, 788. (b) König, W.; Geiger, R. *Ber. Dtsch. Chem. Ges.* **1970**, *103*, 2024, 2034.

(2) (a) Atherton, E.; Cameron, L.; Meldal, M.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1986**, 1763. (b) Jakobsen, M. H.; Buchardt, O.; Engdahl, T.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 6199.

(3) (a) Atherton, E.; Holder, J. L.; Meldal, M.; Sheppard, R. C.; Valerio, R. M. *J. Chem. Soc., Perkin Trans. 1* **1988**, 2887. (b) Cameron, L.; Holder, J. L.; Meldal, M.; Sheppard, R. C. *J. Chem. Soc. Perkin Trans. 1* **1988**, 2895.

(4) Review: Le Nguyen, D.; Castro, B. *Peptide Chemistry 1987. Proceedings of the Japanese Symposium on Peptide Chemistry*; Shiba, T., Sakakibara, S., Eds.; Protein Research Foundation: Osaka, 1988; p 231.

(5) Dourtoglou, V.; Gross, B.; Lambropoulou, V.; Zioudrou, A. *Synthesis* **1984**, 572. Recently, X-ray analysis has shown that the compound to which structure **i** was assigned crystallizes in the form of the corresponding guanidinium salt **5**. A similar structure was established for the analogous 7-aza derivative **ii**. See: Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57. For those compounds for which X-ray data have not yet been obtained (HAPyU, HDTU), the traditional structural representations and nomenclature have arbitrarily been retained.



**i** **ii**

(6) Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927. This work concentrated mainly on the tetrafluoroborate salts. Experimental details for the synthesis of HDTU were not given. Following the method described in ref 5 (Dourtoglou, et al.), HDTU was obtained in 78.9% yield after recrystallization from $CH_3CN/Et_2O$ in the form of white crystals, mp 137–139 °C. $^1H$ NMR ($CD_3CN$): δ 3.4 (s, 12, $CH_3$), 8.1–8.8 (m, 4, aryl). Anal. Calcd for $C_{12}H_{16}F_6N_5O_2P$: C, 35.38; H, 3.93; N, 17.20. Found: C, 35.60; H, 3.93; N, 17.12.

0022-3263/95/1960-3561$09.00/0 

**Table 2. Effect of Identity of Coupling Reagent, Base, and Solvent on the Preservation of Configuration during Formation of Z-Phe-Val-Pro-$NH_2$ via [2 + 1] Coupling[a,b]**

| coupling reagent[c] | base[d] | solvent | yield, % | LDL, % | coupling reagent[c] | base[d] | solvent | yield, % | LDL, % |
|---|---|---|---|---|---|---|---|---|---|
| EDC/HOAt | | DMF | 84.8 | 4.7 | HATU/HOAt | DIEA (3) | DMF | 88.3 | 15.8 |
| EDC/HOAt | | TFE/TCM | 68.8 | 17.9 | HATU/HOAt | TMP (2) | DMF | 72.1 | 2.4 |
| EDC/HODhbt[e] | | DMF | 89.1 | 7.3 | HATU/HOAt | TMP (3) | DMF | 86.8 | 4.5 |
| EDC/HODhbt[e] | | TFE/TCM | 65.8 | 11.4 | HDTU | DIEA (2) | DMF | 87.3 | 10.9 |
| EDC/HOBt | | DMF | 86.7 | 18.9 | HDTU | TMP (2) | DMF | 83.9 | 13.0 |
| EDC/HOBt | | TFE/TCM | 68.7 | 37.8 | HDTU/HODhbt[e] | DIEA (2) | DMF | 76.7 | 4.9 |
| EDC·HCl/HOAt | TMP (1) | DMF | 88.9 | 5.3 | HDTU/HODhbt[e] | DIEA (3) | DMF | 90.2 | 5.5 |
| EDC·HCl/HOAt | TMP (1) | TFE/TCM | 67.8 | 16.9 | HDTU/HODhbt[e] | TMP (2) | DMF | 76.9 | 4.7 |
| EDC·HCl/HODhbt[e] | TMP (1) | DMF | 89.1 | 7.6 | HDTU/HODhbt[e] | TMP (3) | DMF | 88.3 | 5.4 |
| EDC·HCl/HODhbt[e] | TMP (1) | TFE/TCM | 69.8 | 10.2 | HBTU | DIEA (2) | DMF | 89.7 | 27.4 |
| EDC·HCl/HOBt | TMP (1) | DMF | 86.7 | 19.8 | HBTU | TMP (2) | DMF | 81.2 | 14.2 |
| EDC·HCl/HOBt | TMP (1) | TFE/TCM | 65.8 | 36.9 | BOP | DIEA (2) | DMF | 84.1 | 30.4 |
| DCC/HOAt | TMP (1) | DMF | 84.9 | 4.2 | BOP | TMP (2) | DMF | 81.0 | 13.9 |
| DCC/HOAt | TMP (1) | TFE/TCM | 68.9 | 4.6 | HAPyU | DIEA (2) | DMF | 88.9 | 10.8 |
| DCC/HODhbt[e] | TMP (1) | DMF | 88.3 | 12.4 | HAPyU | TMP (2) | DMF | 86.5 | 3.5 |
| DCC/HODhbt[e] | TMP (1) | TFE/TCM | 71.2 | 12.9 | HAPyU/HOAt | DIEA (2) | DMF | 76.8 | 3.2 |
| HATU | DIEA (2) | DMF | 85.8 | 13.9 | HAPyU/HOAt | DIEA (3) | DMF | 90.1 | 12.1 |
| HATU | TMP (2) | DMF | 83.2 | 5.3 | HAPyU/HOAt | TMP (2) | DMF | 75.8 | 1.6 |
| HATU/HOAt | DIEA (2) | DMF | 75.6 | 10.9 | HAPyU/HOAt | TMP (3) | DMF | 89.1 | 2.3 |

[a] For carbodiimide couplings, 0.105 mmol of Z-Phe-Val-OH, 0.1 mmol of H-Pro-$NH_2$, and 0.11 mmol of HOXt were dissolved in 1 mL of DMF or 1.3 mL of TFE/TCM (1:3), and the solution was cooled in an ice bath and treated with 0.11 mmol of EDC, EDC·HCl, or DCC. If a base is added, the number of equivalents is given. The mixture was stirred at 0 °C for 1 h and at room temperature overnight. The mixture was diluted with 25 mL of EtOAc and extracted with 1 N HCl (2 × 5 mL), 1 N $NaHCO_3$ (2 × 5 mL), and saturated NaCl (2 × 5 mL), dried with $MgSO_4$, the solvent was removed, and the crude peptide was directly analyzed by HPLC. For onium salt couplings, 0.125 mmol of the acid, 0.125 mmol of amide, and 0.25 mmol of base in 1 mL of DMF was treated with 0.125 mmol of coupling reagent at 0 °C and the reaction mixture was stirred at 0 °C for 1 h and at room temperature for 2–3 h, after which the workup followed that described above. In cases where an additive is used, one or more equivalents of base (given in parentheses) may be added. If, instead of proline amide, H-Pro-$OCMe_3$·HCl is used in the test for carbodiimide coupling, the results were closely similar to those described in this table, including the fact that extra peaks were noted in the HPLC traces for runs involving HODhbt. Coupling reactions were carried out as described in footnotes *a–d* of this table with workup prior to HPLC analysis following the method in footnote *b*, Table 4 of ref 9. Retention times for the free acids were LLL 8.17 min, LDL 9.96 min. [b] All bases were purified and HPLC analyses carried out as described in footnote *a*, Table 2, ref 9. For HPLC separation, a 4 μm C-18 Waters Nova Pak column (3.9 × 150 mm, 60 Å) was used with a gradient system of 25 to 50% $CH_3CN$ in $H_2O$/0.1% TFA in 25 min, flow rate 1 mL/min, $\lambda_{220\ nm}$, $t_R$(LLL) 14.91 min, $t_R$(LDL) 16.98 min. Authentic samples of the two tripeptide diastereomers were obtained from proline amide and Z-Phe-Val-OH or Z-Phe-D-Val-OH in the normal manner: LLL, mp 168–170 °C; $\alpha^{22}_D = -128.6$ (*c* 0.5, $CHCl_3$); LDL, mp 125–128 °C; $\alpha^{22}_D = -50.2$ (*c* 0.5, $CHCl_3$); $^1H$ NMR, LLL ($CDCl_3$) δ 0.9 (dd, 6, $CH_3$), 1.9–2.2 (m, 5, $CH_2$, CH), 2.9–3.1 (m, 2, $CH_2$), 3.5–3.9 (m, 2, $CH_2$), 4.5–4.9 (m, 5, CH, $CH_2$), 5.11 (d, 2, $CH_2$), 5.6 (d, 1, NH), 6.7 (m, 1, NH), 6.9–7.5 (m, 10, aryl), 7.9 (d, 2, $NH_2$). Anal. Calcd for $C_{27}H_{34}N_4O_5$: C, 65.59; H, 6.88; N, 11.34. Found: C, 65.35; H, 6.86; N, 11.18. [c] EDC was a gift from UBL Scientific, Inc., San Luis Obispo, CA 93401. [d] With EDC, no additional base was added. With other carbodiimides, 1 equiv of base was added per equiv of additive. For onium salts 1 equiv of base is required for activation and a second equivalent is used if an additive is added. In some cases, a third equivalent of base was added, but this is not recommended as it invariably led to increased loss of chirality. The number of equivalents is shown in parentheses. [e] In all cases involving HODhbt and HDTU, extra peaks were observed in the HPLC traces, indicative of side product formation.

workers[7] have demonstrated significant advantages of the mixture EDC/HODhbt in maintaining configurational integrity in the condensation of long segments, especially in the special "structure-breaking" combination solvent trifluoroethanol–trichloromethane (TFE/TCM, 1/3),[7] in which even long peptide segments tend to be soluble.

Because another large category of *N*-hydroxy compounds based on 1-hydroxy-7-azabenzotriazole (HOAt, **3**) more efficient than HOBt has recently been reported,[8] it was of interest to compare these newer reagents with HODhbt under normal conditions and in the special solvent TFE/TCM. Brief mention was earlier made to the greater effectiveness of HOAt over HODhbt in the EDC-based coupling of the sensitive, urethane-protected amino acid Z-Phg-OH with valine methyl ester.[8]

Similar results have now been obtained for the carbodiimide coupling in DMF of Z-Phg-OH to proline amide (Table 1). For this system, coupling yields were uniformly lower (50–70%) in TFE/TCM than in DMF (80–89%). In addition, a more thorough examination of this question is presented for the more important case of peptide segment condensation. Test peptides, in addition to **7** and **8**, both of which had been used previously[9] in

Z–Phe–Val–Ala–OMe (**7**)  Z–Phe–Val–Pro–$OCMe_3$ (**8**)

an extensive comparison of HOAt and HOBt, included **9–11**. For each tripeptide model, the coupling position is shown by the vertical dotted line.

Z–Gly–Phe–Val–OMe[10] (**9**)  Z–Phe–Val–Pro–$NH_2$ (**10**)  Z–Gly–Phe–Pro–$NH_2$ (**11**)

Two of these model peptides involve coupling to proline amide, a feature which is unique for several reasons: (a)

(7) Kurada, H.; Chen, Y.-N.; Kimura, T.; Sakakibara, S. *Int. J. Pept. Prot. Res.* **1992**, *40*, 294. Sakakibara, S. *Biopolymers (Peptide Science)* **1995**, *37*, 17. For selected references on related solvent combinations, see: (a) Toniolo, C.; Bonora, G. M.; Heimer, E. P.; Felix, A. M. *Int. J. Pept. Protein Res.* **1987**, *30*, 232. (b) Narita, M.; Honda, S.; Obana, S. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 342. (c) Narita, M.; Umeyama, H.; Obana, S. *Bull. Chem. Soc. Jpn*, **1988**, *61*, 281. (d) Haver, C. A.; Smith, D. D. *Tetrahedron Lett.* **1993**, *34*, 2239.

(8) Carpino, L. A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.

(9) Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.

(10) (a) Van der Auwera, C.; Van Damme, S.; Anteunis, M. J. O. *Int. J. Pept. Protein Res.* **1987**, *29*, 464. (b) Miyazawa, T.; Donkai, T.; Yamada, T.; Kuwata, S. *Chem. Lett.* **1989**, 2125. The HPLC solvent system used was 40% $CH_3CN$, 60% $H_2O$, 0.1% TFA (isocratic), flow rate 1 mL/min, $\lambda_{220}$ nm, with a 4 μm Nova Pak column (3.9 × 150 mm, 60 Å), $t_R$ (LLL) 12.19 min, $t_R$ (LDL) 13.92 min. Other conditions and instrumentation were as described in footnote *a* of Table 2, ref 9.

**Table 3. Effect of Identity of Coupling Reagent, Base and Solvent on the Preservation of Configuration during Formation of Z-Phe-Val-Ala-OMe via [2 + 1] Coupling[a,b]**

| coupling reagent | base | solvent | yield, % | LDL, % |
|---|---|---|---|---|
| EDC/HOAt | | DMF | 81.9 | 0.7 |
| EDC/HOAt | | TFE/TCM | 74.3 | <0.1 |
| EDC/HODhbt[c] | | DMF | 82.6 | 0.1 |
| EDC/HODhbt[c] | | TFE/TCM | 74.6 | <0.1 |
| EDC/HOBt | | DMF | 82.3 | 3.3 |
| EDC/HOBt | | TFE/TCM | 76.1 | <0.1 |
| EDC·HCl/HOAt | TMP (1) | DMF | 80.9 | 0.5 |
| EDC·HCl/HOAt | TMP (2) | DMF | 89.8 | 0.5 |
| EDC·HCl/HOAt | TMP (1) | TFE/TCM | 82.7 | <0.1 |
| EDC·HCl/HODhbt[c] | TMP (1) | DMF | 83.1 | 0.6 |
| EDC·HCl/HODhbt[c] | TMP (2) | DMF | 92.9 | <0.1 |
| EDC·HCl/HODhbt[c] | TMP (1) | TFE/TCM | 84.4 | <0.1 |
| EDC·HCl/HOBt | TMP (1) | DMF | 85.6 | 3.3 |
| EDC·HCl/HOBt | TMP (2) | DMF | 90.1 | 3.5 |
| EDC·HCl/HOBt | TMP (1) | TFE/TCM | 81.1 | <0.1 |
| DCC/HOAt | TMP (2) | DMF | 93.5 | 0.2 |
| DCC/HOAt | TMP (2) | TFE/TCM | 92.7 | <0.1 |
| DCC/HODhbt[c] | TMP (2) | DMF | 97.9 | 0.1 |
| DCC/HODhbt[c] | TMP (2) | TFE/TCM | 96.9 | <0.1 |
| DCC/HOBt | TMP (2) | DMF | 96.1 | 3.4 |
| DCC/HOBt | TMP (2) | TFE/TCM | 96.3 | 0.8 |
| HATU | DIEA (3) | DMF | 82.3 | 1.9 |
| HATU | TMP (3) | DMF | 81.3 | <0.1 |
| HDTU[c] | DIEA (3) | DMF | 89.3 | 2.5 |
| HDTU[c] | TMP (3) | DMF | 89.1 | 0.6 |
| HBTU | DIEA (3) | DMF | 81.9 | 6.0 |
| HBTU | TMP (3) | DMF | 80.3 | 2.9 |
| BOP | DIEA (3) | DMF | 81.2 | 3.2 |
| BOP | TMP (3) | DMF | 80.1 | 1.8 |

[a] Coupling reactions were carried out as described in footnotes *a*–*d*, Table 2, except that, since the amino acid ester was added as the hydrochloride, an extra equivalent of base was used. The total number of equivalents of base is given. For other examples involving this tripeptide see refs 8 and 9. [b] For HPLC separation, a 4 $\mu$m C-18 Waters Nova Pak column (3.9 × 150 mm, 60 Å) was used with an isocratic system of 40% $CH_3CN$/60% $H_2O$/0.1% TFA, flow rate 1 mL/min, $\lambda_{220}$ nm, $t_R$(LLL) 13.78 min, $t_R$(LDL) 16.05 min. Other conditions and instrumentation were as described in footnote *a* of Table 2, ref 9. [c] In these cases, extra peaks were observed in the HPLC traces, indicative of side product formation.

greater sensitivity toward loss of chirality[11] due to both steric effects and the greater basicity of proline relative to other amino acids;[12] (b) availability of proline amide as a stable base rather than a hydrochloride, which would require addition of an extra equivalent of a tertiary amine in order to liberate the free base; (c) greater ease of separation by HPLC of diastereomeric peptides bearing a C-terminal amide function.

For segment couplings leading to tripeptides, the relative merits of HOAt or HODhbt depend on the identity of the two amino acids at which coupling occurs. Results are collected in Tables 2 and 3. For the case of Z-Phe-Val-Pro-$NH_2$, **10**, carbodiimide couplings in the presence of HOAt proved to be generally somewhat more efficient than those of HODhbt except in TFE/TCM where the reverse was true (Table 2). However, the differences were not large, and results in DMF were generally better than in TFE/TCM. For tests involving onium salts, only DMF was used as solvent since, as expected, TFE reacts with the onium salt, thereby rendering it inactive. For the similar case of Z-Phe-Val-Pro-$OCMe_3$, used earlier[9] in testing bases of varied degrees of steric hindrance around the nitrogen atom, the results were closely analogous to those observed with Z-Phe-Val-Pro-$NH_2$ (see

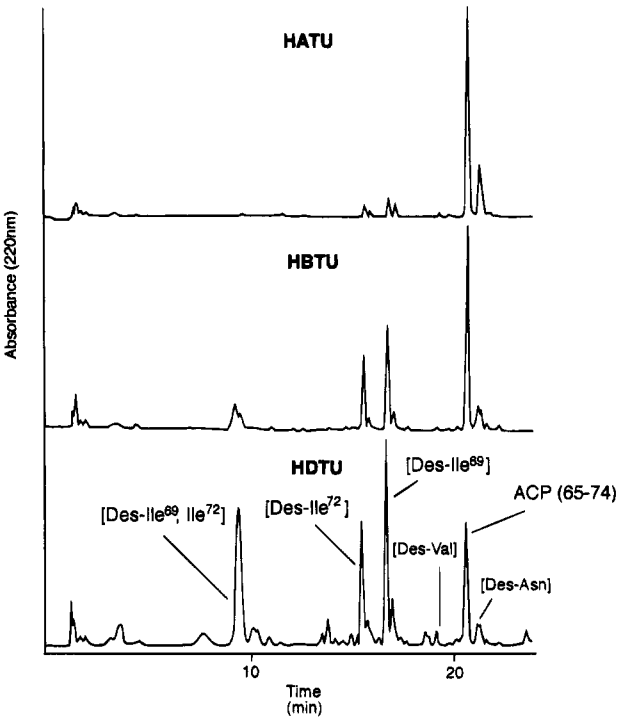



**Figure 1.** Comparison of HPLC traces of ACP **12**, assembled via HATU, HBTU, and HDTU. All syntheses were carried out on a Biosearch continuous flow 9050-Plus instrument using 1.5 equiv each of the amino acid and coupling reagent, 3 equiv of DIEA, a preactivation time of 7 min, and a coupling time of 1.5 min (compare ref 16).

footnote *a* of Table 2). When the same model was transferred to solid phase, *i.e.*, coupling of Z-Phe-Val-OH to H-Pro-NH-PEG-PS in DMF, the same deleterious effect of extended preactivation time was observed, as recorded previously for another case.[13] Thus, for HATU/TMP and preactivation times of 5, 2, and 0 min, the amount of the LDL form was 20.5, 12.2, and 9.4%, respectively; for HDTU/TMP the corresponding figures were 43.9, 12.5, and 8.3%; for HBTU/TMP, the figures were 21.4, 20.5, and 13.5%, and for HAPyU/TMP they were 15.9, 14.0, and 8.8%.

For Z-Phe-Val-Ala-OMe, **7**, also used earlier[9] in tests of various bases, HODhbt was generally more efficient than HOAt for carbodiimide couplings in DMF (Table 3). The efficiency of the Sakakibara solvent for this system is emphasized by the fact that all three additives including HOBt were equally effective. However, for onium salt couplings, HATU was superior to HDTU, HBTU, or the BOP reagent.

The system Z-Gly-Phe-Val-OMe,[10] **9**, proved to be rather insensitive, and for carbodiimide couplings, <0.1% of the LDL form was observed in all cases (comparable to runs listed in Table 2) except when HOBt was used as an additive (*e.g.*, EDC/HOBt/DMF, 0.9% LDL; EDC·HCl/HOBt/TMP (2)/DMF, 1.0% LDL). In TFE/TCM, all carbodiimide couplings (HOAt, HODhbt, and HOBt) gave <0.1% of the LDL form. On the other hand, for onium salt couplings, epimerization levels up to 5.3% of LDL were noted according to the order HAPyU <HATU < HDTU < HBTU < BOP, with only HAPyU/TMP (3) allowing coupling with formation of less than 0.1% of the LDL form.

(11) Itoh, M.; Nojima, H.; Notani, J.; Hagiwara, D. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 3320.
(12) Sakakibara, S.; Itoh, M. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 656.
(13) Carpino, L. A.; El-Faham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279.

Finally, with system Z-Gly-Phe-Pro-$NH_2$,[14] **11**, coupling in DMF via carbodiimide reagents gave comparable retention of configuration for both HOAt and HODhbt. In the case of onium salts, HATU and HDTU gave similar results (1.3–6% LDL form) and again only with HAPyU/HOAt/TMP (2) was it possible to bring the level of epimerization to less than 0.1%.

It is curious that several exceptions to the generally observed[9,13] superiority of collidine over DIEA as activating base were noted: (a) HDTU in three cases (Z-Gly-Phe-Val-OMe, Z-Phe-Val-Pro-$NH_2$, Z-Gly-Phe-Pro-$NH_2$) and (b) the BOP reagent in two cases (Z-Gly-Phe-Val-OMe, Z-Gly-Phe-Pro-$NH_2$). This inversion was not observed for any system which incorporated HOAt, HATU, or HAPyU. Considering the data listed in Tables 1–3, as well as the related data discussed above for systems **8, 9**, and **11**, where a direct comparison is made between HOAt- and HODhbt-derived reagents, in over half of the cases, HOAt systems proved more effective. If the comparison is limited to cases involving DMF as solvent or collidine as base, the advantage of HOAt-derived reagents was even higher, whereas for carbodiimide couplings in TFE/TCM, HODhbt and HOAt systems were about equally effective. For reasons not yet clear, collidine as base appears to be particularly suitable for systems derived from HOAt. It may also be noted that the most efficient way of using the HAPyU–collidine combination is by addition of 1 equiv each of HOAt and collidine.

The generalizations offered here apply only to the test systems described.[15] Additional examples are under study for the coupling of segments terminating and beginning in other amino acids, especially longer segments and those bearing more sensitive C-terminal proteinogenic amino acids such as histidine or cysteine. Although the use of HOAt and HODhbt in segment coupling offers significant improvement over analogous reactions involving HOBt, examination of the data in Tables 1–3 emphasizes that the goal of developing rapid, racemization-free segment coupling processes, applicable to peptide bond formation between *any* two amino acids, remains elusive.

Special effects may govern the application of these systems to solid phase coupling reactions. For solid phase systems, the question of preactivation time[13] is of utmost importance, and to date, fully appropriate techniques for safe coupling in solvents such as DMF are not available. It is interesting to note that, in spite of its efficiency in the preservation of chirality as observed previously and confirmed here, the use of HODhbt (in the form of the onium salt HDTU) for automated, stepwise solid phase synthesis is not satisfactory. Thus, for the synthesis of the common test decapeptide ACP,[16] **12**, whether under normal or forcing conditions, HDTU does not provide a significant yield of the desired peptide (Figure 1).

H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-$NH_2$
**12**

**Acknowledgment.** We are indebted to the National Science Foundation (NSF CHE-9003192), the National Institutes of Health (GM-09706), and the Millipore Corporation for support of this work. The National Science Foundation is also thanked for grants used to purchase the high-field NMR spectrometers used in this research. Mr. Byron Bossenbroek of Chemical Abstracts Service is thanked for advice regarding the revised nomenclature of some of the onium salts.

JO950037F

(14) Coupling reactions were carried out as described in footnotes *a–d*, Table 2. The tripeptide had mp 87–89 °C; $\alpha^{23}_D = -38.6$ ($c = 0.5$, $CHCl_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 1.8–2.2 (m, 4, $CH_2$), 2.9–3.1 (m, 2, $CH_2$), 3.6–4.0 (m, 4, $CH_2$), 4.5 (m, 1, CH), 4.9 (m, 1, CH), 5.12 (s, 2, $CH_2$), 5.8 (m, 1, NH), 6.3 (m, 1, NH), 6.9–7.4 (m, 12, aryl, NH). Anal. Calcd for $C_{24}H_{28}N_4O_5 \cdot CH_3OH$: C, 61.98; H, 6.61; N, 11.57. Found: C, 62.01; H, 6.55; N, 11.64. The HPLC separation was worked out from a sample of the mixed tripeptides (mp 94–96 °C) derived from Z-Gly-Phe-OH and H-DL-Pro-$NH_2$, using a gradient system 25 to 50% $CH_3CN/H_2O$, 0.1% TFA in 25 min, flow rate 1 mL/min, $\lambda_{220\ nm}$, $t_R$ (LL) 10.2 min, $t_R$ (DL) 11.7 min. Instrumentation, columns, etc., were as described in footnote *a* of Table 2, ref 9.

(15) The specific effects of the two amino acids between which coupling occurs have been emphasized in earlier studies. See: Benoiton, N. L. in *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1983; Vol. 5, p 217.

(16) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201.